



Ultraviolet-B effects on stomatal density, water-use efficiency, and stable carbon isotope discrimination in four glasshouse-grown soybean (*Glycine max*) cultivars

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Abstract

Interactions between UV-B radiation and drought stress have been studied but the underlying mechanisms have not been thoroughly investigated. We hypothesized that UV-B radiation would improve water-use efficiency (WUE) by its effects on epidermal development, specifically stomatal density, and leaf gas exchange. Four lines of soybean (*Glycine max*: Essex, Williams, OX921, and OX922) were grown for 28 days in a glasshouse with and without supplemental UV-B radiation levels of 13 kJ m⁻² biologically effective radiation. UV-B radiation increased phenolic content of leaves in all lines and reduced leaf area in all lines except Williams. Adaxial stomatal density was reduced in Essex, OX921, and OX922 but abaxial stomatal density was reduced only in OX922. Stomatal conductance was reduced in concert with stomatal density as was internal CO₂ concentration (C_i). Instantaneous WUE was increased in Essex, Williams, and OX922. Stable carbon isotope analysis showed similar trends in long-term WUE, but these were only statistically significant in OX922. These results suggest that some soybean cultivars may respond to increased levels of UV-B by increasing WUE and that this response could be manifested through changes in stomatal development and functioning. Field studies are needed to test this hypothesis and to further evaluate the role of the *K9* gene in this response.

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Keywords: Flavonoids; Stomata; UV-B; Water-use efficiency

1. Introduction

Continued depletion of stratospheric ozone by anthropogenic (Anderson et al., 1991) and natural (McCormick et al., 1995) halogens may result in further increases in the levels of ultraviolet-B radiation (UV-B, between 280 and 320 nm) reaching the earth's surface. Although the UV-B waveband is a small

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fraction of the total terrestrial solar spectrum, UV-B radiation elicits disproportionately large responses from some plants while others appear to be tolerant to even high levels of UV-B. In general, plant responses to UV-B might be arbitrarily divided into two classes, photomorphogenic and stress responses. Stress responses are characterized by the damage leading to reductions in metabolic processes such as photosynthesis, plant growth, or seed yield in crops that result from an applied or increased level of a given stressor as compared to a plant's typical growing conditions (Lichtenthaler, 1995). Photomorphogenic responses on the other hand, result in altered architecture or chemical composition and may be thought to be adaptive responses of plants to the incident radiation micro-climate (Beggs and Wellman, 1994; Ballaré et al., 1992).

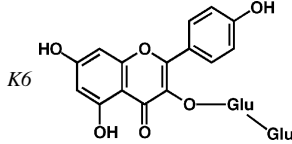
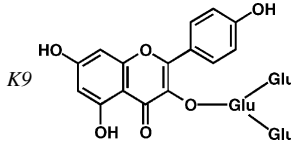
Since it appears likely that biomass or productivity is not dramatically altered by moderate increases in UV-B radiation, it is of particular interest to evaluate the role of UV-B radiation in altering plant photomorphogenesis. For example, indirect effects of UV-B radiation on ecosystem structure and functioning may be of equal or greater importance than direct effects on plant productivity (e.g. Caldwell and Flint, 1994; Sullivan, 1997; Sullivan and Rozema, 1999). These effects may include alterations in competitive interactions and ecosystem composition (Gold and Caldwell, 1983; Barnes et al., 1996) as well as those that alter the functioning of ecosystems such as plant–insect or plant–pathogen interactions (e.g. Lavola, 1998; McCloud and Berenbaum, 1994) and nutrient decomposition (Gehrke et al., 1995; Newsham et al., 1999; Cybulski et al., 2000).

The response of plants to UV-B radiation can be altered by concurrent changes in other micro-climatic factors such as PAR (e.g. Warner and Caldwell, 1983; Mirecki and Teramura, 1984; Cen and Bornman, 1990), nutrient status (Murali and Teramura, 1985), and water stress (Murali and Teramura, 1986; Sullivan and Teramura, 1990; Nogués et al., 1998). In general, it has been reported that the effects of UV-B radiation are masked in the presence of nutrient deficiency or drought. On the other hand, it has also been suggested that exposure to UV-B might partially alleviate drought stress due to changes in epidermal anatomy or wax deposition (Petropoulou et al., 1995; Laakso et al., 2000), reduction in leaf area (Nogués et al.,

1998), or increases in the stomatal limitation to carbon assimilation (Sullivan and Teramura, 1990; Middleton and Teramura, 1993; Allen et al., 1998; Nogués et al., 1999). However, Nogués and Baker (2000) did not find that exposure of three Mediterranean plants to UV-B radiation before or during drought had an effect on the response of these species to drought. Therefore, while an interaction between UV-B and drought has been observed in some species (e.g. Sullivan and Teramura, 1990; Nogués et al., 1998), the mechanism for this interaction has not been elucidated.

The purpose of this study was to investigate whether and how responses to UV-B radiation would lead to enhanced water-use efficiency (WUE) in soybean. We selected four soybean lines for this study: Williams, Essex, OX921, and OX922. Williams and Essex have been fairly well described and used as UV-B tolerant and susceptible cultivars, respectively; based on the effects of UV-B radiation on growth and development, yield, and UV-screening compound accumulation (Teramura and Murali, 1986; Lydon et al., 1986). The isolines OX921 and OX922 were selected because of their unique flavonoid composition, differences in stomatal development, and differential response of stomatal development to blue light (Liu-Gitz et al., 2000), which could lead to differential responses to UV-B radiation. The soybean isolines differ by a single gene based upon phenotypic segregation in breeding experiments (Buzzell et al., 1980). Their phenotypic characteristics are summarized in Table 1. Within the OX921–OX922 pair, OX922 is almost entirely hypostomatous and produces a kaempferol glycoside with a uniquely branched tri-glucosyl side chain (K9). OX922 also has reduced chlorophyll content, photosynthetic rate, and stomatal conductance compared to its sister isolate, i.e. OX921 (Buzzell et al., 1980). OX921 is amphistomatous and has a typical complement of kaempferol glycosides relative to many soybean cultivars. It was hypothesized that K9 directly inhibits stomatal formation in OX922 (Buzzell et al., 1980). It has also been shown that stomatal development in OX922 is inhibited by blue light and that this response is separable from a phytochrome response (Liu-Gitz et al., 2000). In this study, our working hypothesis was that photomorphogenic changes in epidermal development by UV-B would result in reduced stomatal density and lead to altered gas exchange characteristics and enhanced WUE in soybean.

Table 1
Comparison of selected characteristics of soybeans isolines OX921 and OX922

	OX921	OX922
Genotype	fg ₁ fg ₂ Fg ₃ Fg ₄	Fg ₁ fg ₂ Fg ₃ Fg ₄
Predominant flavonol glycoside		
Stomatal density (mm ⁻²)		
Upper	130–160	3–5
Lower	300–340	140–240

Adapted from Buttery and Buzzell (1987, 1992).

2. Materials and methods

2.1. Plant material, growth conditions, and irradiation treatments

Soybean (*Glycine max* L. Merr.) plants (cv. Williams, Essex, OX921, and OX922) were grown in the glasshouse in 2-L pots (10 pots per cv.) on sterilized soil for five weeks with or without supplemental UV-B radiation. To produce plants of uniform size and vigor, five seeds were planted in each pot and thinned to a single plant by severing the stem at the surface of the soil after primary leaves were expanded. Direct sunlight in the glasshouse at mid-day represented the maximal potential insolation and was predominately dependent on the transmittance of the glazing (>90% of ambient). Daylong integrated PAR was about 50% of ambient due to shading by the lamps and the greenhouse structural elements. UV-B radiation from fluorescent sunlamps was supplied essentially according to Teramura et al. (1990). Briefly, supplemental UV radiation was delivered to plants for 8 h centered around mid-day by UV-B-313 fluorescent lamps (Q-Panel Co., Cleveland, Ohio) which were arranged in two 12-lamp racks (1.2 m × 3.05 m, 1 × W) driven by 40 W dimming ballasts. To filter out UV-C radiation (<280 nm) and short-wave UV-B (<290 nm) not absorbed by the lamp envelope, the lamps were wrapped with pre-solarized 0.08 mm cellulose diacetate film (Cadillac Plastic, Pensauken, NJ). Energy delivered to the plants was checked daily with a broadband radiometer (Model 3D UV Meter, Solar Light Co. Inc., Philadelphia, PA) that had been calibrated to our lamps' spectral distribution

with an OL754 spectroradiometer calibrated with an OL752-10 NIST traceable 200 W tungsten–halogen standard lamp (Optronic Laboratories, Inc., Orlando, FL). Rack height, lamp spacing, and lamp power were adjusted as needed to maintain a total daily flux of biologically effective UV radiation of 13 kJ m⁻² (Caldwell, 1971). This relatively high level of UV-B, which corresponds to the maximum mid-summer and clear sky irradiance that would be anticipated with a 25% ozone depletion over Beltsville, MD according to an empirical model (Green et al., 1980), was selected to correspond with other studies on soybean (e.g. Murali and Teramura, 1985, 1986) rather than to attempt realistic simulation of field conditions. Control plants were illuminated by lamps wrapped with polyester film spectrally equivalent to Mylar type D plastic, which blocks both UV-B and UV-C. Since the greenhouse glass absorbs strongly in the UV-B waveband, the control plants received no UV-B radiation. The spectral distributions of lamp and filter combinations are compared to sunlight within the glasshouse in Fig. 1. Plants were rotated under the lamp banks every third day in an attempt to minimize potential effects resulting from micro-environmental variation. All measurements were made on the central trifoliate leaflet of the fourth leaf (>1 cm) from the apex, unless otherwise noted.

2.2. Pigment analysis

For phenolic analysis, leaf disks (diameter = 1.35 cm) were taken and placed in 20 ml high density polyethylene (HDPE) scintillation vials, covered with 10 ml of slightly acidified aqueous methanol

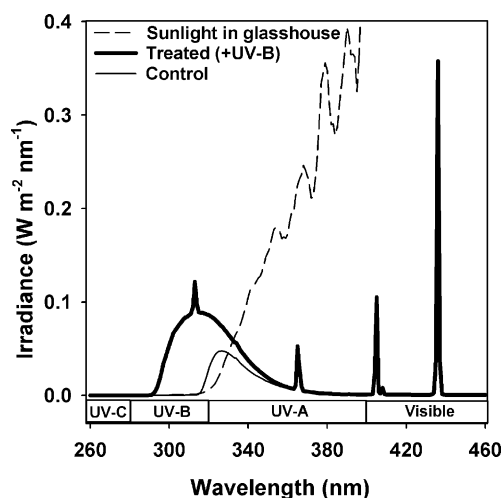


Fig. 1. Spectral distribution of supplemental radiation delivered by UV-B-313 lamps wrapped with polyester film (control: light solid line) or cellulose diacetate film (UV-B: heavy solid line). Measurements were made with an Optronics OL-754 spectroradiometer after sundown for clarity. Spectral distribution of sunlight in the glasshouse is shown for comparison (broken line). UV treatments were delivered for 8 h centered around solar noon. Lamps were adjusted to deliver daily exposure levels of 13 kJ m^{-2} of biologically effective ultraviolet-B radiation (Caldwell, 1971).

(methanol:water:acetic acid; v:v:v; 50:50:1), tightly capped and held in the dark at room temperature with gentle agitation (50 rpm) for 48–72 h. During extraction, vials were sealed with polyethylene lined caps, since it was found that foil lined caps would occasionally corrode and contaminate the extracts leading to the non-uniform formation of metal–flavonol complexes and bathochromic shifting of the absorbance spectra. To insure that photosynthetic pigments were not present in the phenolic extracts, the extract absorbance was determined from 260 to 760 nm at 1-nm intervals with a Shimadzu UV-1601 Spectrophotometer dual beam spectrophotometer. For presentation, data from 260 to 500 nm are shown.

Chlorophyll content was determined by extraction of leaf disks in ethanol essentially as described by Knudson et al. (1977) with simultaneous estimation of carotenoids content as described by Jaspers (1965).

2.3. Leaf morphology

Leaf blades were removed from the petiole and area was determined using a LI-3000 portable area meter.

Stomatal density (stoma mm^{-2}) of abaxial and adaxial leaf surfaces was determined using cyanoacrylate leaf impressions made on glass slides (Wilson et al., 1986). Stoma were counted along transects mid-way between the mid-rib and leaf edge and parallel to the mid-rib (Gitz, 1993; Liu-Gitz et al., 2000).

2.4. Gas exchange measurements

Gas exchange measurements were made with a Li-C or LI-6400 portable photosynthesis system fitted with a LI-6400-02B LED light source that uses mixed LEDs delivering both red and blue light to leaves in the sample cuvette. In an attempt to minimize temporal variation, only five plants of each line were selected from each treatment to insure that measurements could be completed between mid-morning and mid-afternoon. During measurement, the system maintained a constant $1800 \mu\text{mol m}^{-2} \text{ s}^{-1}$ PAR, 25°C leaf temperature, and 50% R.H. Instantaneous WUE was determined as moles of CO_2 assimilated/moles of H_2O transpired at ambient CO_2 ($C_a = 350 \mu\text{mol mol}^{-1}$). The PAR level used was selected by trial, in which it was determined that all of the lines had reached maximal photosynthetic rates at this level. The leaves were allowed to acclimate in the instrument until conditions within the sample cuvette had stabilized and assimilation had reached an apparent steady state, typically 5–10 min. Stomatal limitation was determined by elevating the CO_2 concentration within the sample cuvette, allowing the system to stabilize, and making observations at 350, 400, 450, and $500 \mu\text{mol mol}^{-1} \text{ CO}_2$. The assimilation rate (A) was plotted against leaf internal CO_2 concentration (C_i), and assimilation at ambient CO_2 ($A_{C_i} = C_a$) was estimated by linear regression of the four data points of the A – C_i curve. Stomatal limitation to carbon assimilation was calculated as $1 - (A_{C_a}/A_{C_i} = C_a)$, where A_{C_a} is assimilation at ambient CO_2 ($350 \mu\text{mol mol}^{-1}$) (Farquhar and Sharkey, 1982).

Conductance measurements were made on both upper and lower leaf surfaces with a LI-1600 steady state porometer (Li-Cor Inc., Lincoln, NE) on the same plants used for WUE, and stomatal limitation determinations (above). In the interest of safety, plants were removed from the bench beneath the sunlamps and arranged along adjacent empty benches in the evening prior to making measurements. This made plants more

accessible and enhanced sampling speed, which was also a consideration since light and temperature variations through the day can affect leaf conductance and introduce systematic temporal errors. Since plants did not receive supplemental radiation immediately prior to or during conductance measurements, any UV-B effects observed were considered to have been cumulative rather than instantaneous.

2.5. Stable carbon isotope analysis

The above-ground portion of the plants was oven dried at 60 °C and weighed to determine shoot biomass. The dried shoots were ground to pass through a 60 mesh screen and 2–3 mg portions placed into tin capsules for measurement of $\delta^{13}\text{C}$ abundance ratios on a SIRA Series II mass spectrometer (Micromass, Manchester, UK) at the Duke University Phytotron. Reference CO_2 was calibrated against standard Pee

Dee belemite (Ozteck, Dallas TX). For presentation, data were converted from $\delta^{13}\text{C}$ to Δ , the stable carbon isotope fractionation resulting from the assimilation weighted intercellular CO_2 concentration during the lifetime of the plant (Farquhar et al., 1989).

2.6. Statistical analysis

Previous studies have shown that soybean cultivars may differ in the magnitude of response to UV-B radiation (e.g. Murali and Teramura, 1986; Lydon et al., 1986) and that OX921 differs developmentally from OX922 (Buzzell et al., 1980). In the present study, a two-way ANOVA verified that there were significant response differences among lines (e.g. significant species-by-treatment interactions). Therefore, we evaluated the presence or absence of a UV-B effect within each line by the use of two-tailed student's *t*-tests. Probability levels of rejecting the

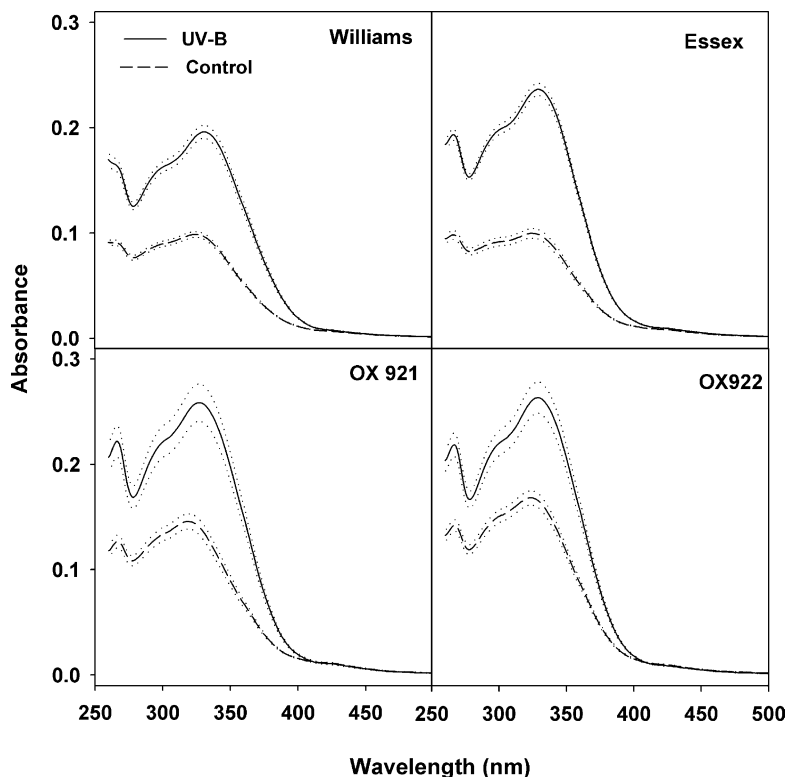


Fig. 2. Mean absorbance of 50% aqueous methanolic leaf disk extracts from soybean plants grown with (UV-B) or without (control) supplemental UV-B radiation. Values are means ($n = 10 \pm \text{S.E.}$). Differences between experimental treatments were significant at each measured wavelength below 400 nm for every cultivar ($P < 0.001$, *t*-test).

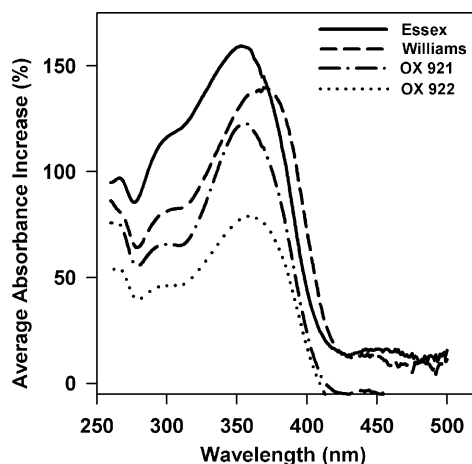


Fig. 3. Mean increase (%) in absorbivity of 50% aqueous methanolic leaf disk extracts under UV-B radiation as compared to plants grown under control conditions. Data are from Fig. 2.

null hypothesis are shown in each case and unless indicated otherwise, our discussion assumes significance at $P < 0.05$. The number of samples measured is specified in the text, tables and figures. All experiments were reproduced at least twice with similar results, with the exception of stable carbon isotope analysis which was done only once. All results presented here were from the experimental run which included this data-set.

3. Results

When plants were grown under supplemental UV-B, the absorbance of leaf phenolic extracts increased in all lines as compared to plants grown under polyester wrapped lamps (Fig. 2). In every case the increase in absorbance was highly significant ($P < 0.001$, student's t -test) at each wavelength within the UV-B waveband and throughout much of the UV-A waveband. However, there were differences between lines in the absolute levels of phenolics and in the proportional response to UV-B radiation (Fig. 3).

The maximal absorption of the extracts was at around 330 nm, however, the relative increase in extract absorbivity was greatest between 360 and 380 nm. Although there were differences in the degree of absorbance enhancement at different wavelengths, the curves appear generally very similar (Fig. 3).

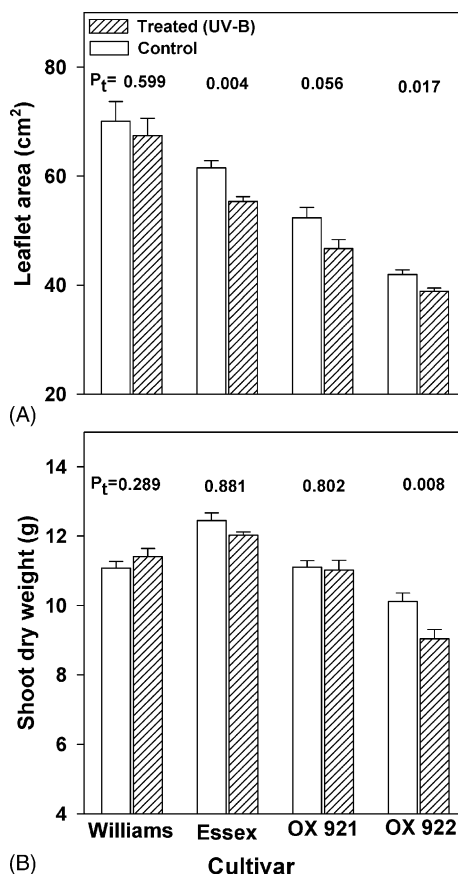


Fig. 4. Area of central trifoliate leaflet (A) and above ground biomass (B) of soybean plants grown with (shaded bars) and without (open bars) supplemental UV-B radiation. In each case bars are means \pm S.E. ($n = 10$) and results of a two-tailed (student's) t -test are shown (P_t).

This suggests that it would have been adequate to measure leaf extract absorbance at any wavelength within the UV-B spectrum, as long as other absorbing pigments that might confound measurement were not present in the extracts. In this case absorbance maxima characteristic of photosynthetic pigments were not detected. Although putative UV-screening compounds increased, leaf area was reduced in all lines except Williams (Fig. 4A). Shoot biomass, however, was only reduced in the OX922 isoline (Fig. 4B).

Photosynthesis, as measured by CO_2 assimilation, was also only reduced by UV-B radiation in OX922 but transpiration was reduced in all lines except OX921 (Table 2). This was accompanied by a

Table 2
Effect of UV-B on selected leaf gas exchange parameters in glasshouse-grown soybean leaves

		Williams	Essex	OX921	OX922
Assimilation ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Control	27	25.9	25.7	22.6
	UV-B	25.7	25.5	26.4	14.2
	P_t	ns	ns	ns	0.0001
Transpiration ($\text{mmol m}^{-2} \text{s}^{-1}$)	Control	14.34	13.00	12.12	8.46
	UV-B	12.34	11.194	12.24	4.06
	P_t	0.012	0.006	ns	<0.0001
C_i (ppm)	Control	295	290.2	288	257.8
	UV-B	285.2	278.8	285.4	215.6
	P_t	0.044	0.005	ns	0.001

Plants were grown under sunlamps wrapped with either polyester (control) or cellulose diacetate film (UV-B). Values are means ($n = 5$) and P_t is the result of a two-tailed t -test; ns indicates $P_t > 0.1$.

lower internal CO_2 concentration in these same lines (Table 2).

In general, UV-B did not consistently affect photosynthetic pigments (Table 3). However, total chlorophyll showed a trend toward an increase under supplemental UV-B in Essex whereas UV-B reduced the Chl a:Chl b ratio and the ratio of carotenoids to chlorophylls in OX922 (Table 3).

Adaxial stomatal density was significantly reduced by UV-B radiation in all lines except Williams, which showed a similar trend ($P = 0.086$) but abaxial stomatal density was only reduced in the OX922 line (Fig. 5A and C). Adaxial conductance was not measurable in OX922, but in the other lines conductance (by steady state porometry) exhibited a pattern similar to that of stomatal density (Fig. 5A and C), as did abaxial conductance in OX922 (Fig. 5B and D). The stomatal limitation to photosynthesis, instantaneous WUE, and above ground carbon stable isotope discrimination

(Δ) were affected to various degrees across lines by UV-B radiation (Fig. 6A–C). The almost exclusively hypostomatous OX922 was the most responsive to supplemental UV-B radiation. In that isoline, the calculated stomatal limitation to assimilation and WUE were increased by UV-B radiation (Fig. 6A and B). Also, plant ^{13}C discrimination (Fig. 6C) and calculated long-term C_i from these data (224 to $202 \mu\text{mol mol}^{-1}$ for the control and UV-B treatments, respectively) were reduced by UV-B radiation, consistent with instantaneous observations (Table 2). On the other hand, even though adaxial density and conductance were reduced by UV-B radiation, these changes did not lead to alterations in stomatal limitation, WUE, or Δ in OX921. Instantaneous WUE and stomatal limitations were increased by UV-B in Essex and Williams (but note that $P = 0.05$ for stomatal limitation in Williams) but these changes did not lead to changes in carbon isotope discrimination.

Table 3
Effect of UV-B on photosynthetic pigments (chlorophylls and carotenoids) in glasshouse grown soybean leaves

	Cultivar	Williams	Essex	OX921	OX922
Chl a+b (mg cm^{-2})	Control	42.14	42.92	47.45	37.54
	UV-B	43.54	44.73	46.91	35.32
	P_t	ns	0.072	ns	ns
Chl a/b	Control	3.67	3.69	3.57	3.61
	UV-B	3.58	3.66	3.48	3.31
	P_t	ns	ns	ns	<0.001
Car/Chl	Control	0.195	0.197	0.184	0.190
	UV-B	0.189	0.195	0.179	0.175
	P_t	ns	ns	ns	0.003

Plants were grown under sunlamps wrapped with either polyester (control) or cellulose diacetate film (UV-B). Means and P_t , the results of two-tailed t -test are shown ($n = 7$); 'ns' indicates $P_t > 0.1$.

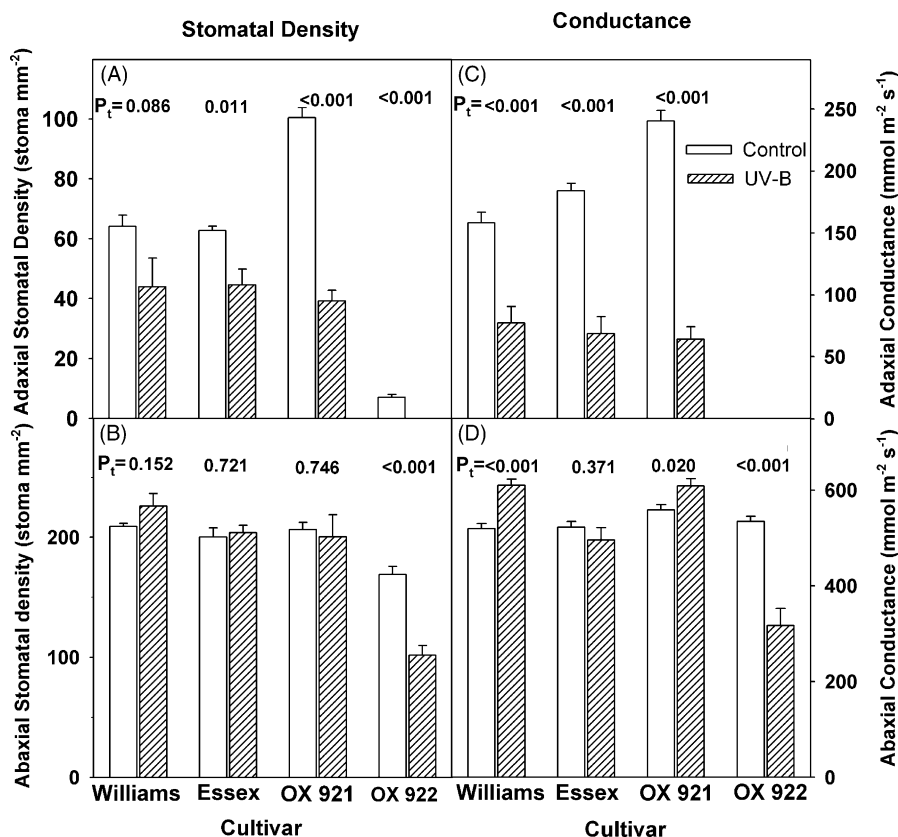


Fig. 5. Stomatal density and conductance in soybean leaves grown with (shaded bars) and without (open bars) supplemental UV-B radiation. Left: Adaxial (upper graph, A) and abaxial (lower graph, B) stomatal density of soybean leaves, $n = 5$. Results of two-tailed (student's) t -test are shown. Right: Adaxial (upper, C) and abaxial (lower, D) stomatal conductance. Adaxial stomatal conductance of OX922 was not measurable with available instrumentation, $n = 10$. In each case bars are means \pm S.E. and results of two-tailed (student's) t -test are shown (P_t).

4. Discussion

An increase in phenolics in response to UV-B radiation is a response that is frequently assayed and consistently reported in soybean and other plant species (e.g. Beggs and Wellman, 1994; Bornman, 1999). In fact, in a recent meta analysis, Searles et al. (2001) found that an increase in UV-absorbing compounds in response to supplemental UV-B was the most consistently reported response to UV-B radiation. In this study, we used a rapid assay of leaf extracts to indicate the potential for a UV response, and to insure that plants received effective levels of UV radiation. If increases in phenolics pigments are elicited through mechanisms similar to that of architectural responses

(Larkin et al., 2003), then one might view phenolics accumulation as a surrogate measure for potential induction of the entire suite of putative UV photomorphogenic responses. In this case, all lines demonstrated a UV-B radiation-induced increase in phenolics that was consistent with those obtained in field studies in which soybean was grown under enhanced UV-B (e.g. Murali and Teramura, 1986; Sullivan and Teramura, 1990), or with ambient UV-B selectively removed by growth under cutoff filters (Schumaker et al., 1997; Mazza et al., 2000). The maximal absorption was consistent with hydroxycinnamic acids (HCAs) being the predominate UV absorbing compounds in the extracts (Swain, 1976). However, the relative increase in extract absorptivity was greatest between 360 and

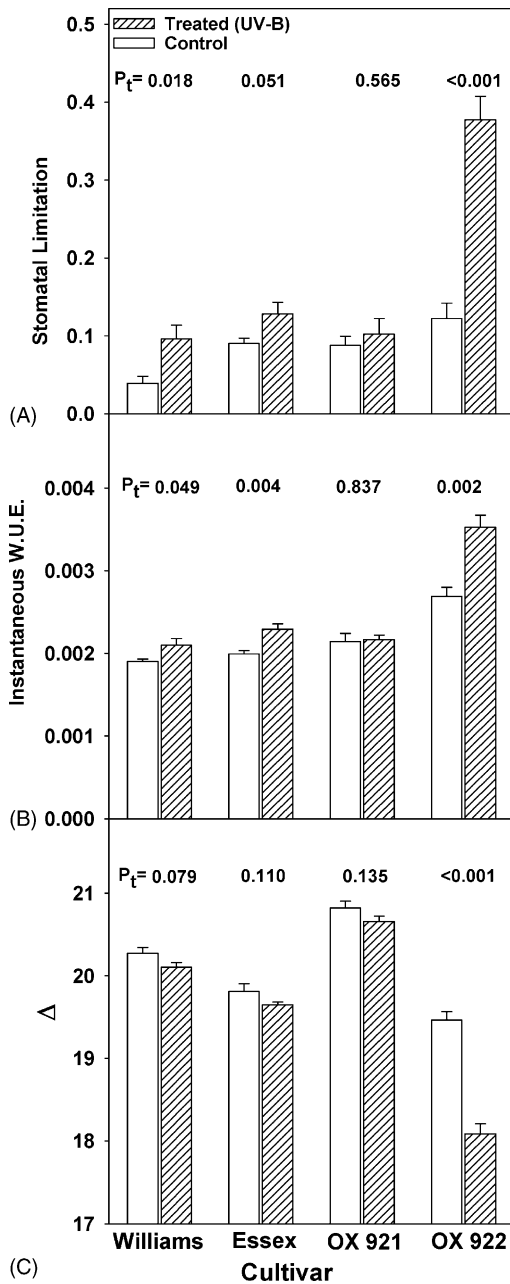


Fig. 6. Gas exchange parameters and stable carbon isotope discrimination of soybean grown with (shaded bars) or without (Control, open bars) supplemental UV-B radiation: (A) stomatal limitation; (B) instantaneous WUE; and (C) stable carbon isotope fractionation (Δ). Bars are means \pm S.E., $n = 5$. Results of two-tailed (student's) t -test are shown.

380 nm, which is in the range of flavonol absorption (Markham, 1982). Subtle differences in the baseline and UV-inducible levels of phenolics could contribute to the differences in responses to UV-B radiation among cultivars of soybean. For example, in this study OX922 exhibited about half the increases in phenolics noted in the other lines and it was the only line that showed any reduction in carbon assimilation by UV-B radiation. These results are also parallel to those of other studies on soybean that have shown response differences among cultivars (e.g. Biggs et al., 1981; Teramura and Murali, 1986; Reed and Teramura, 1992).

Given the differential morphological and physiological responses among and between species and the subtle nature of these responses, the focus of this study was to evaluate whether UV-B effects on stomatal development or gas exchange would lead to improved WUE. Adaxial stomatal density was depressed in all lines, consistent with the direct action of UV upon the developing epidermal structures. However, it remains to be conclusively shown whether the observed reductions in stomatal density are the result of UV damage to developing stomatal initials or the result of a true photomorphogenic (developmental) process. A generalized inhibition of epidermal cell division by UV has been reported in greenhouse grown pea, although stomatal densities were generally unaffected (Nogués et al., 1998). A reduction in stomatal density in response to blue light during leaf development was reported in OX922, caused by arrested stomatal development primarily at the guard mother cell stage (Liu-Gitz et al., 2000). When leaf impressions from the present study were examined, it was found that similar developmentally inhibited cell complexes could be found on the adaxial surface of all UV-B irradiated leaves (not shown), so the possibility of a direct effect of UV-B on development exists and should be further evaluated. Since leaf area was decreased or unchanged in all lines, the reduction in stomatal density was not a result of effects of UV-B on leaf expansion (e.g. a dilution of stomatal density due to expanded leaf area). Distribution of the same number of stomata over a smaller surface would have increased, rather than decreased stomatal density, and comparable effects would have been expected on both leaf surfaces.

Stomatal conductance generally exhibited a response pattern similar to that of the reductions in

stomatal density, especially on the adaxial leaf surface. Nogués et al. (1999) found that reductions in conductance in pea (*Pisum sativum*) plants exposed to high levels of UV-B were largely the result of altered stomatal (guard cell) functioning rather than of stomatal density. In this study, changes in stomatal aperture could have also played a role in the lower conductance observed but the marked decrease in adaxial stomatal density appears to have played a role as well. It is also possible that UV-B radiation could alter conductance from a residual UV-B effect upon stomatal physiology leading to altered responsiveness even though the plants were removed from beneath the lamps for measurement. For example, in OX922, which is almost exclusively hypostomatous, abaxial conductance was also reduced. Interestingly, abaxial stomatal conductance was increased in Williams and OX921. Since these leaf surfaces received little direct UV-B radiation as compared to the upper surfaces, it is possible that this was a compensatory indirect increase in conductance that reduced the overall effects of UV-B on C_i and WUE in Williams compared to OX922.

The effects of UV-B radiation on stomatal conductance were, except for OX922, independent of apparent direct effects on assimilation. The absence of apparent photosynthetic damage in the present study is not uncommon in UV-B studies. In fact, this result parallels several recent studies where direct damage by UV-B was not found (e.g. Nogués et al., 1998, 1999; Nogués and Baker, 2000). While UV-B can directly damage photosynthesis if present in sufficient quantities; it appears that these plants were protected from photosynthetic damage, perhaps by the observed increases in phenolics (see Sullivan and Rozema, 1999 for a review). Although these data suggest a trend of a general response in the same direction, it was clear that the response to UV-B was enhanced in the OX922 isoline. Reduction of photosynthesis was observed only in this isoline and this could have been due to the apparently lower response of protective phenolics to changes in photosynthetic pigments, as well as stomatal limitation. It is unknown whether the presence of K9 itself was related to this response or due to pleiotropic consequences of the K9 gene such as changes in other pigments or the hypostomatous structure. The reduction in the ratio of chl a:b and the ratio of carotenoids to chlorophyll could also

have led to this enhanced sensitivity of photosynthesis. Middleton and Teramura (1993, 1994) reported increases in carotenoids in response to UV-B, but it is unclear whether this provides any direct photoprotection from UV-B since carotenoids exhibit little absorbance in this waveband. If the effects reported were simply due to direct effects on adaxial stomata then one might predict that the OX922 isoline would be least responsive since it has very few adaxial stoma and virtually no adaxial conductance. However, since OX922 appeared to be most sensitive line, in terms of parameters measured in this study, it appears that indirect or systemic effects led to the responses observed.

It has been hypothesized that UV signal perception can lead to photomorphogenic responses that may confer adaptive advantages under conditions associated with high-light environments such as water stress (Gitz and Liu-Gitz, 2003). Alteration in stomatal conductance in the absence of reduced assimilation could enhance instantaneous WUE or increase the stomatal limitation to photosynthesis. This has been demonstrated in some cases (e.g. Sullivan and Teramura, 1990; Teramura et al., 1990; Middleton and Teramura, 1993; Dai et al., 1995; Allen et al., 1998; Nogués et al., 1999) and could lead to improved drought tolerance. Also, several other researchers have reported anatomical changes that could lead to improved water relations upon exposure to UV-B radiation. For example, increased cutinization was found in Mediterranean evergreens (Manetas et al., 1997; Bjorn et al., 1997) and in Scots pine (*Pinus sylvestris*) (Laakso et al., 2000). Increased epidermal cell wall thickness was also found in loblolly pine (*Pinus taeda*) and Scots pine (Laakso et al., 2000). These changes could also improve tolerance to drought. However, Nogués and Baker (2000) found no effect of UV-B on the drought response in three Mediterranean species grown under enhanced UV-B radiation before and during the imposition of drought treatments. Also, reductions in transpiration, conductance, and C_i with UV-B have been reported in soybean, but altered carbon isotope ratios were attributed to photosynthetic disruption (Feng et al., 2003).

This study is one of the few that documented changes in both stomatal density and gas exchange leading to increases in instantaneous and long-term WUE (^{13}C isotope discrimination). The responses

were most pronounced in the OX922 isoline. We conclude that while reductions in stomatal density by UV-B could lead to changes in drought tolerance of soybean, the magnitude of such effects are clearly cultivar dependent. The potential for the responses to occur under field conditions needs to be thoroughly evaluated. Identification of the underlying mechanisms would be useful in determining the role of the K9 genotype in shaping or acting as a marker for altered stomatal distribution and the sensitivity of stomatal development to UV-B.

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